



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12P 7/64, A23D 9/013		A1	(11) International Publication Number: WO 99/06585 (43) International Publication Date: 11 February 1999 (11.02.99)
(21) International Application Number:	PCT/US98/15835		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date:	31 July 1998 (31.07.98)		
(30) Priority Data:	60/054,563	1 August 1997 (01.08.97)	US
(71) Applicant (<i>for all designated States except US</i>):	MARTEK BIOSCIENCES CORPORATION [US/US]; 6480 Dobbin Road, Columbia, MD 21045 (US).		
(72) Inventors; and			
(75) Inventors/Applicants (<i>for US only</i>):	GLADUE, Raymond, M. [US/US]; 655 Brookgreen Lane, Lexington, KY 40509 (US). BEHRENS, Paul, W. [US/US]; 4725 Widdup Court, Ellicott City, MD 21043-6529 (US).		
(74) Agents:	POSORSKE, Laurence, H. et al.; Baker & Botts, LLP., The Warner, 1299 Pennsylvania Avenue, N.W., Washington, DC 20004 (US).		
Published <i>With international search report.</i>			

(54) Title: DHA-CONTAINING NUTRITIONAL COMPOSITIONS AND METHODS FOR THEIR PRODUCTION

(57) Abstract

This invention provides a particulate material suitable for use as a nutritional supplement, particularly as an aquaculture feed. The particulate material has a high proportion of DHA residues in the lipid fraction, which may be up to 35% of the material, or even more. Preferably, the material has a mean particle size of from about 5 microns to about 10 microns. This invention also provides a method for preparing a particulate material suitable for use as an aquaculture feed from microbial biomass, preferably from algal cells having a high content of DHA residues, by obtaining a lipid fraction from the biomass, preferably by solvent extraction of broken cells, followed by separating a fraction containing phospholipids and proteins from the lipid fraction, and removing water from the protein/phospholipid fraction to form a low moisture particulate, preferably by spray-drying the protein/phospholipid fraction.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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EE	Estonia						

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 98/15835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P7/64 A23D9/013

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 130 242 A (PHYCOTECH, INC.) 14 July 1992 cited in the application see the whole document ---	1-24
X	US 5 397 591 A (MARTEK BIOSCIENCES CORPORATION) 14 March 1995 cited in the application see the whole document	9-13
Y	US 5 492 938 A (MARTEK BIOSCIENCES CORPORATION) 20 February 1996 cited in the application see the whole document	1-8, 14-24
X	US 5 492 938 A (MARTEK BIOSCIENCES CORPORATION) 20 February 1996 cited in the application see the whole document	9-13
Y	US 5 492 938 A (MARTEK BIOSCIENCES CORPORATION) 20 February 1996 cited in the application see the whole document	1-8, 14-24
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"S" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

22 October 1998

29/10/1998

Name and mailing address of the ISA

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Douschan, K

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 98/15835

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 407 957 A (MARTEK CORPORATION) 18 April 1995 see the whole document	9-13
Y	---	1-8, 14-24
X,P	US 5 711 983 A (MARTEK BIOSCIENCES CORPORATION) 27 January 1998 see the whole document	9-13
A,P	WO 98 03671 A (NAGASE BIOCHEMICALS LTD. AND SUNTORY LIMITED) 29 January 1998 see the whole document	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat'l Application No

PCT/US 98/15835

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 5130242	A 14-07-1992	AT 156520 T		15-08-1997
		AU 657259 B		09-03-1995
		AU 6719590 A		13-06-1991
		CA 2072978 A		18-05-1991
		DE 69031223 D		11-09-1997
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US 5397591	A 14-03-1995	US 5407957 A		18-04-1995
		US 5492938 A		20-02-1996
		US 5711983 A		27-01-1998
		AT 165212 T		15-05-1998
		AU 660162 B		15-06-1995
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INTERNATIONAL SEARCH REPORT

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Internat Application No

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Patent document cited in search report	Publication date	Patent family member(s)			Publication date
US 5492938	A	CA	2076018	A	14-08-1991
		DE	69129296	D	28-05-1998
		EP	0515460	A	02-12-1992
		ES	2116288	T	16-07-1998
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		JP	5503425	T	10-06-1993
		WO	9111918	A	22-08-1991
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			AU	660162	B
			AU	7330291	A
			CA	2076018	A
			DE	69129296	D
			EP	0515460	A
			ES	2116288	T
			IL	97126	A
			IL	111174	A
			JP	5503425	T
			WO	9111918	A
			US	5492938	A
			US	5711983	A
			US	5397591	A
US 5711983	A	27-01-1998	US	5407957	A
			AT	165212	T
			AU	660162	B
			AU	7330291	A
			CA	2076018	A
			DE	69129296	D
			EP	0515460	A
			ES	2116288	T
			IL	97126	A
			IL	111174	A
			JP	5503425	T
			WO	9111918	A
WO 9803671	A	29-01-1998	AU	2979897	A
					10-02-1998

PATENT COOPERATION TREATY

REC'D 11 NOV 1999

PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 062308.0290	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US98/15835	International filing date (day/month/year) 31 JULY 1998	Priority date (day/month/year) 01 AUGUST 1997
International Patent Classification (IPC) or national classification and IPC IPC(6): C12P 7/64; A23D 9/13; C07C 69/52 and US Cl.: 435/134; 560/205; 426/601		
Applicant MARTEK BIOSCIENCES CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 25 JANUARY 1999	Date of completion of this report 20 SEPTEMBER 1999
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer JON F. WEBER, PH.D. Telephone No. (703) 308-0196
Facsimile No. (703) 305-3230	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/15835

I. Basis of the report

1. This report has been drawn on the basis of (Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments):

- the international application as originally filed.
- the description, pages 1-16, as originally filed.
pages NONE, filed with the demand.
pages NONE, filed with the letter of _____.
pages , filed with the letter of _____.
- the claims, Nos. 1-24, as originally filed.
Nos. NONE, as amended under Article 19.
Nos. NONE, filed with the demand.
Nos. NONE, filed with the letter of _____.
Nos. , filed with the letter of _____.
- the drawings, sheets/fig NONE, as originally filed.
sheets/fig NONE, filed with the demand.
sheets/fig NONE, filed with the letter of _____.
sheets/fig , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

- the description, pages NONE.
- the claims, Nos. NONE.
- the drawings, sheets/fig NONE.

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the **Supplemental Box Additional observations below (Rule 70.2(c))**.

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/15835

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. STATEMENT**

Novelty (N)	Claims <u>1-24</u>	YES
	Claims <u>NONE</u>	NO
Inventive Step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-24</u>	NO
Industrial Applicability (IA)	Claims <u>1-24</u>	YES
	Claims <u>NONE</u>	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-24 lack an inventive step under PCT Article 33(3) as being obvious over Phycotech, Inc. (US 5,130,242) in view of Martek Biosciences Corporation (US 5,397,591) and further in view of KILBRIDE, JR. et al (US 5,000,888) and COLE et al (US 5,104,668).

Phycotech, Inc. (US 5,130,242) disclose making DHA from *Thraustochytriales* by extracting the organisms, extracting with hexane, saponifying, extracting again, and low temperature fractional crystallization. The refining methods are said to be conventional in the art. Feed supplements for aquaculture, humans or poultry, for example, may be prepared by mixing the crude oil with feed.

Martek Biosciences Corporation (US 5,397,591) disclose that dinoflagellates and especially *Cryptocodonium cohnii* are excellent sources of DHA-containing oil (30-40% of the triglycerides are DHA and other components such as the polar lipids - phosphatidyl choline - are also enriched in DHA). The DHA-containing oil is obtained by harvesting the organisms, extracting with hexane or more directly with polar solvents such as ethanol or isopropanol, and then removal of the solvent. The crude oil may be processed by conventional means in the vegetable oil processing art to allow separation of the DHA-enriched polar lipid fractions. The resulting oil may be added to infant formulas (homogenization needed) or infant foods, which are more solid in constitution.

KILBRIDE, JR. et al (US 5,000,888) disclose that it is conventional in the spray drying art to control the spray drying conditions with biological samples so as to obtain a desired particle size. Conditions can be controlled for stability of the biological samples as well.

Salt Lake Brine Shrimp, Inc. (US 5,104,668) disclose that it is desirable in the aquaculture art to provide feeds having a particle size in the range of 5 to 50 microns for the purpose of feeding shrimp and fish. The particles are prepared by spray drying a homogenate of the desired seedstock.

A person of ordinary skill in the art would be motivated to prepare DHA containing oils in dried form of 5-10 microns for the purpose of aquaculture or other feeds such as infant formula (Continued on Supplemental Sheet.)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/15835

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 10 and 15-18 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s):

Claim 10 recites "a particulate" which should be two words.

Claims 15-18 depend from claim 1, which lacks antecedent basis. It is believed that these claims should depend from claim 14.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

V. 2. REASoNED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
according to the teachings of Phycotech, Inc. (US 5,130,242) and Martek Biosciences Corporation (US 5,397,591) because drying biologicals by spray drying is conventional in the art as shown by KILBRIDE, JR. et al (US 5,000,888) and because COLE et al (US 5,104,668) show that it is known in the art that spray dried feed particles suitable for aquaculture should be from 5 to 50 microns. Providing an aqueous homogenate of the DHA-containing oil is reasonably suggested by the infant formula of Martek Biosciences Corporation (US 5,397,591). The spray dried form is reasonably suggested by COLE et al (US 5,104,668).

Claims 1-24 meet the criteria for novelty and industrial applicability set out in PCT Articles 33(2) and (4), because the prior art does not teach spray dried 5-10 micron particles of polar extracts of DHA-containing organisms. Several uses of the particles in feedstocks and pharmaceuticals are suggested.

----- NEW CITATIONS -----

US 5,000,888 A (KILBRIDE, JR. et al) 19 March 1991, see columns 3-7.

US 5,104,668 A (COLE et al) 14 April 1992, see Fig. 1 and columns 2-3.

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)
16 February 1999 (16.02.99)

International application No.	Applicant's or agent's file reference
PCT/US98/15835	062308.0298
International filing date (day/month/year)	Priority date (day/month/year)
31 July 1998 (31.07.98)	01 August 1997 (01.08.97)

Applicant
GLADUE, Raymond, M. et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

25 January 1999 (25.01.99)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 062308.0298	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 98/ 15835	International filing date (day/month/year) 31/07/1998	(Earliest) Priority Date (day/month/year) 01/08/1997
Applicant MARTEK BIOSCIENCES CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of **3** sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Certain claims were found unsearchable (see Box I).
2. Unity of invention is lacking (see Box II).
3. The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
 - filed with the international application.
 - furnished by the applicant separately from the international application,
 - but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
 - Transcribed by this Authority
4. With regard to the title, the text is approved as submitted by the applicant
 - the text has been established by this Authority to read as follows:
5. With regard to the abstract,
 - the text is approved as submitted by the applicant
 - the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is:

Figure No. _____

 - as suggested by the applicant.
 - because the applicant failed to suggest a figure.
 - because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/15835

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12P7/64 A23D9/013

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P A23D

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Further documents are listed in the continuation of box C.

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- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"&" document member of the same patent family

Date of the actual completion of the international search

22 October 1998

Date of mailing of the international search report

29/10/1998

Name and mailing address of the ISA

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Authorized officer

Douschan, K

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/15835

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A,P	WO 98 03671 A (NAGASE BIOCHEMICALS LTD. AND SUNTORY LIMITED) 29 January 1998 see the whole document -----	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/15835

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5130242	A	14-07-1992	AT 156520 T AU 657259 B AU 6719590 A CA 2072978 A DE 69031223 D DE 69031223 T DK 512997 T EP 0512997 A ES 2106736 T GR 3025200 T JP 8509355 T WO 9107498 A US 5518918 A US 5656319 A US 5688500 A US 5698244 A US 5340594 A US 5340742 A	15-08-1997 09-03-1995 13-06-1991 18-05-1991 11-09-1997 05-03-1998 02-02-1998 19-11-1992 16-11-1997 27-02-1998 08-10-1996 30-05-1991 21-05-1996 12-08-1997 18-11-1997 16-12-1997 23-08-1994 23-08-1994
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